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# EFFECT OF ETHANOL EXTRACT OF *ZIZIPHUS MAURITIANA* LEAF ON SERUM AND HEPATIC LIPID PEROXIDATION AND VITAMIN C LEVELS IN ACUTE ALCOHOL TREATED RATS

**D. DAHIRU AND M.N. EJIOFOR**

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## ABSTRACT

This study evaluated the effect of pretreatment with ethanol extract of *Ziziphus mauritiana* leaf on serum and hepatic lipid peroxidation and vitamin C in alcohol-induced liver damage. Elevated levels of serum enzyme markers alanine aminotransferase (ALT) aspartate aminotransferase (AST) ALP) were observed in alcohol group compared to normal rats. Also total bilirubin and lipid peroxidation increased significantly ($p<0.05$) in alcohol group compared to normal control. Pretreatment with ethanol extract of *Ziziphus mauritiana* leaf 50, 100, 200 mg/kg body weight (bw) for 5 days significantly ($p<0.05$) decreased raised levels of serum enzyme markers of liver tissue damage and protected rats against serum and hepatic lipid peroxidation compared to group administered alcohol only. The extract of *Ziziphus mauritiana* was also observed to significantly reduce the depletion of vitamin C compared to group administered alcohol only. The effect of the extract was observed to be dose-dependent.


**KEYWORDS:** Lipid peroxidation, *Ziziphus mauritiana*, alcohol, vitamin C. Corresponding author: ddahiru2000@yahoo.com

## INTRODUCTION

Focus on plant research has increased all over the world and a large body of evidence exists to show immense potential of medicinal plants used in various traditional systems (Dahanukar *et al.*, 2000). Although important progress has been made in understanding the pathogenesis of alcoholic liver disease, current therapies for this disease are not effective. Novel therapeutic approaches such as utilizing agents that successfully correct the fundamental cellular disturbances resulting from excessive alcohol consumption are attractive (Zhou *et al*, 2002). The toxic effects of alcohol are directly related to the plasma levels achieved after alcohol intake. Practically, all the alcohol absorbed is metabolized in the liver where it undergoes two oxidative processes during which it is first converted into acetaldehyde, its toxic metabolite, and then to acetate. As a consequence of the hepatic metabolism of alcohol series of processes leading to liver damage take place (Caballeria, 2003). In the liver, ethanol toxicity may be associated with elevated production of reactive oxygen intermediates. Lipid peroxidation is a major consequence of oxidative stress leading to tissue damage (Saravanan, 2003). The identification of naturally occurring inhibitors of peroxidation resulting in cell damage could therefore lead to important new strategies for disease prevention (Subramonian *et al.*, 1999).

*Ziziphus mauritiana* Lam. belongs to the family *Rhamnaceae*. It is widely grown in mild-temperate, rather dry areas, of both hemispheres and is adapted to warm climates. It is often called jujube, Chinese date, and Indian plump (Morton, 1987). In northern Nigeria, it is called magarya (Hausa) or huya (Kilba). The plant finds various uses in traditional medicine. For instance; the fruits are applied on cuts and ulcers; are employed in pulmonary ailments and fevers; the dried ripe fruit is a mild laxative. The seeds are sedative and are taken sometimes with butter, to halt nausea, vomiting and abdominal pains in pregnancy. They check diarrhoea, and are applied on wounds. Mixed with oil, they are rubbed on rheumatic areas. The leaves are helpful in liver disease, asthma and fever. The bitter, astringent bark decoction is taken to halt diarrhoea and dysentery and relieve gingivitis. A root decoction is given as a febrifuge and the powdered root is dusted on wounds. Juice of the root bark is said to alleviate gout and rheumatism (Morton, 1987 and Michel, 2002). The root is also used in the treatment of epilepsy (Msonthi *et al.*, 1983).

This research investigated the effect of ethanol extract of Ziziphus mauritiana leaf on serum and hepatic lipid peroxidation in acute alcohol-induced hepatotoxicity.

## MATERIALS AND METHODS

### Animals

Male albino rats, weighing between 130 and 150g were obtained from the Veterinary Research Institute Vom, Jos, Plateau State, and were housed in a well-ventilated room under 12h light/dark cycle. The animals were fed with a commercial diet (Vital Feed: Grand Cereals and Oil Mill Ltd, Jos) and water *ad libitum*.

### Preparation of plant material

The plant material, *Ziziphus mauritiana* leaves (fresh) were collected 20km along Yola-Mubi road in the month of July 2005. The leaves were dried under room temperature at 30 ± 2°C. The dried material was made into powder using mortar and piston and sieved with Endicott test sieve 0.3mm (Endicott Ltd, London). A voucher specimen of the plant has been deposited (BCDD-03b) in the Department of Biochemistry Federal University of Technology, Yola. The extract was obtained from the powder (100g) through Soxhlet extraction with 90% methanol. The extract was concentrated into a semi-solid material using rotary evaporator at < 50°C. The extract was dissolved in distilled water to 50 or 100, and 200 mg/ml and administered to rats orally.

### Hepatotoxin

Absolute was purchased from Mallinckrodt Chemical Works St. Louis, USA. The ethanol was Analar (99.8%). A 5g/kg body weight of 25% w/v alcohol solution was administered as the hepatotoxin.

### Animal treatment

Thirty-six male rats were divided into six groups of 6 animals each. Group I normal control; group II alcohol control; group III; group IV and V, were pretreated with single daily dose of 50, 100 and 200 mg/kg body weight of *Ziziphus mauritiana* extract respectively for 5 days followed by daily single dose of 5g/kg body weight of 25% w/v alcohol solution

**D. Dahiru,** Department of Biochemistry, School of Pure and Applied Sciences, Federal University of Technology, Yola P. M. B. 2076 Yola, Adamawa State, Nigeria.

**M.N. Ejiofor,** Department of Biochemistry, School of Pure and Applied Sciences, Federal University of Technology, Yola P. M. B. 2076 Yola, Adamawa State, Nigeria.

for 3 days. Group VI extract control, received only extract for 3 days (200 mg/kg body weight).

**Collection of blood and tissue samples**

At the end of the experimental period, the animals were sacrificed after ether anesthesia and blood collected without the use of anti-coagulant for serum preparation. The blood collected was allowed to stand for 10 min before being centrifuged at 2,000 rpm for 10 min after which the serum was separated from the plasma using a rubber micropipette and used for the analysis. The levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin (Bil) were assayed using Randox clinical test kits (Randox Laboratories, Ltd U.K.); lipid peroxidation (TBARS) was according to Wills (1987) while vitamin C was determined as described (Roe, 1973). The liver was excised after dissecting the animals with a dissecting blade and placed in ice-cold saline in a beaker. Weighed amount of liver tissue was minced and homogenized in Tris HCl buffer solution (1g liver tissue/10ml) using a glass Teflon, motorized Potter-Elveghem homogenizer (Melsungen, Germany). The homogenate was then centrifuged at 3000g for 10min at 4°C and 0.2ml supernatant used for tissue lipid peroxidation assay.

**Statistical Analyses**

Results were presented as Mean ± standard error of mean. Statistical analyses was carried out using student't' test to compare for significant difference between two means. Significant difference was considered at $p<0.05$ with the aid of Microsoft Data Analysis. Microsoft Excell 2003.

**RESULTS**

Table 1 shows the levels of enzyme markers and non-enzyme maker of tissue damage in groups treated with alcohol only, with *Ziziphus mauritiana* and in normal rats. Enzyme markers included AST, ALT, and ALP while non-enzyme marker is Bilirubin. The levels of assayed enzyme markers were significantly ($p<0.05$) increased in group administered alcohol only (GP II) compared to normal rats (GP I). Groups pre-treated with ethanol extract of *Ziziphus mauritiana* (50, 100, and 200 mg/kg body weight) showed significant ($p<0.05$) decrease in levels of assayed enzyme markers of liver damage compared with group treated with alcohol only. Also level of bilirubin in alcohol control significantly ($p<0.05$) increased compared to normal rats. The level of bilirubin in groups pre-treated with the extract however, significantly ($p<0.05$) decreased compared to alcohol control group.

Table 2, shows the effect of ethanol extract of *Ziziphus mauritiana* leaf on levels of serum lipid peroxidation (TBARS) and serum vitamin C on alcohol-induced liver damage. The levels of lipid peroxidation as assayed by thiobabituric acid reactive substances significantly ($p<0.05$) increased in alcohol control compared to normal rats. Significant ($p<0.05$) decrease in the level of serum lipid peroxidation was observed in groups pretreated with ethanol extract of *Ziziphus mauritiana* leaf (50, 100 and 200 mg/kg body weight). Significant ($p<0.05$) decrease in the level of serum vitamin C was also observed in alcohol control group when compared to normal rats. However pretreatment with 100 and 200 mg/kg body weight of ethanol extract of *Ziziphus mauritiana* significantly ($p<0.05$) raised the serum vitamin C level of the respective groups.

Table 3 shows the effect of ethanol extract of Ziziphus mauritiana leaf on hepatic lipid peroxidation and vitamin C. Significant ($p<0.05$) decrease in hepatic lipid peroxidation was observed in groups pretreated with the extract while significant increase in hepatic vitamin C was observed in groups pretreated with 100 and 200 mg/kg body weight.

**Table 1:** Effect of ethanol extract of *Ziziphus mauritiana* leaf on serum biochemical markers of liver damage

| Treatment | AST (IU/L) | ALT (IU/L) | ALP (IU/L) | BIL (µmol/L) |
|---|---|---|---|---|
| Normal rats (GP I) | 83 ± 0.55 | 320 ± 3.63 | 501 ± 2.0 | 23.10 ± 1.3 |
| Alcohol only (GP II) | 178 ± 0.32$^{\alpha}$ | 625 ± 5.12 $^{\alpha}$ | 827 ± 2.5 $^{\alpha}$ | 68.15 ± 0.1 $^{\alpha}$ |
| 50 mg/kg bw Zm + alcohol (GP III) | 152 ± 0.84$^{\beta}$ | 520 ± 4.16 $^{\beta}$ | 660 ± 3.3 $^{\beta}$ | 59.10 ± 0.1 $^{\beta}$ |
| 100 mg/kg bw Zm + alcohol (GP IV) | 134 ± 0.63$^{\beta\gamma}$ | 480 ± 3.18 $^{\beta\gamma}$ | 650 ± 6.8 $^{\beta\gamma}$ | 30.00 ± 0.4 $^{\beta\gamma}$ |
| 200 mg/kg bw Zm + alcohol (GP V) | 94 ± 0.88$^{\beta\gamma\delta}$ | 340 ± 6.77 $^{\beta\gamma\delta}$ | 620 ± 3.0 $^{\beta\gamma\delta}$ | 22.70 ± 0.2 $^{\beta\gamma\delta}$ |
| 200 mg/kg bw Zm only (VI) | 88 ± 0.45 | 350 ± 1.1 | 530 ± 2.3 | 24.80 ± 0.6 |

Values are Means ± Standard deviation, (n = 6). α statistically significant ($p<0.05$) compared to GP I; β significantly lower ($p<0.05$) compared to GP II; γ significantly ($p<0.05$) lower relative to GP III; δ significant ($p<0.05$) relative to GP IV.

Table 2: Effect of ethanol extract of *Ziziphus mauritiana* leaf on serum lipid peroxidation and vitamin C levels

| Treatment | TBARS (nmol/h) | Vitamin C (mg/100ml) |
|---|---|---|
| Normal rats (GP I) | 26.8 ± 0.32 | 0.295 ± 0.01 |
| Alcohol only (GP II) | 38.4 ± 0.14 $^{\alpha}$ | 0.290 ± 0.03 |
| 50 mg/kg bw Zm + alcohol (GP III) | 31.7 ± 0.22 $^{\beta}$ | 0.302 ± 0.08 |
| 100 mg/kg bw Zm + alcohol (GP IV) | 30.0 ± 0.71$^{\beta}$ | 0.324 ± 0.05 $^{\beta}$ |
| 200 mg/kg bw Zm + alcohol (GP V) | 28.3 ± 0.55$^{\beta\gamma}$ | 0.328 ± 0.06 $^{\beta}$ |
| 200 mg/kg bw Zm only | 27.3 ± 0.63 | 0.300 ± 0.07 |

Values are Means ± Standard deviation, (n = 6). α significantly higher ($p<0.05$) compared to GP I; β significantly lower ($p<0.05$) compared to GP II; γ significantly ($p<0.05$) lower relative to GP III.

Table 3. Effect of ethanol extract of *Ziziphus mauritiana* leaf on hepatic lipid peroxidation and vitamin C levels

| Treatment | TBARS (nmol/h) | Vitamin C (mg/100g) |
|---|---|---|
| Normal rats (GP I) | 2.07 ± 0.10 | 0.832 ± 0.031 |
| Alcohol only (GP II) | 7.12 ± 0.04$^{a}$ | 0.516 ± 0.052$^{a}$ |
| 50 mg/kg bw Zm + alcohol (GP III) | 6.04 ± 0.14$^{b}$ | 0.621 ± 0.038 |
| 100 mg/kg bw Zm + alcohol (GP IV) | 4.13 ± 0.08$^{b}$ | 0.683 ± 0.025$^{b}$ |
| 200 mg/kg bw Zm + alcohol (GP V) | 3.81 ± 0.17$^{b}$ | 0.815 ± 0.043 $^{b}$ |
| 200 mg/kg bw Zm only | 2.21 ± 0.12 | 0.854 ± 0.036 |

Values are mean ± Means ± Standard deviation, (n = 6); $^{a}p<0.05$ significantly higher compared to control; $^{b}p<0.05$ significantly lower compared to alcohol only.

## DISCUSSION

In the past, liver disease was the major concern associated with alcoholism, and was believed to be primarily due to poor dietary habits. Consequently, treatment was limited to addressing overt nutritional deficiencies. While malnutrition occurs in varying degrees in almost all alcoholics, subsequent research showed that alcohol itself is directly hepatotoxic (Amber *et al.*, 2000). The animals treated with alcohol only (GP II) resulted in a significant hepatic damage as elicited by the elevated levels of serum enzyme markers of liver damage and bilirubin. The rise in the ALT level is usually accompanied by an elevation in the levels of AST, which plays a role in the conversion of amino acids to keto acids (Sallie, 1999). The CYP2E1 has a collectively high redox potential, which, on using $NADP^+$ as a cofactor, leads to the formation of free oxygen radical, oxidative stress and lipid peroxidation. Oxidative stress induces the activation of kupffer cells, increasing the expression of several cytokines such as transforming growth factor beta, tumor necrosis factor alpha and interleukin-1. All of these processes contribute to the activation of stellate cells with the consequent increase in collagen synthesis favoring the progression of alcoholic liver disease (Caballeria, 2003). Pretreatment with the extract of *Ziziphus mauritiana* attenuated a decreased level of the serum enzymes and bilirubin in all the pretreated groups, thus suggesting that the extract at all the doses given were able to condition the hepatocytes in order to protect the membrane integrity against alcohol-induced leakage of enzymes into the circulation (Lin, 2000). Alcohol ingestion is known to promote oxidative stress in mammals and humans. An increase in the free radical production is likely to play a role in the induction of severe liver damage (Saravanan, 2003). In the present study, it was observed that there was an increase in serum and hepatic lipid peroxidation as assayed by TBARS in alcohol group (GP II). This indicates that there was an activation of the lipid peroxidation system in both serum and liver tissue. Lipid peroxidation is initiated by the abstraction of a hydrogen atom from the side chain of polyunsaturated fatty acids in the membrane lipids (Saravanan, 2003). The high vulnerability of neutral tissue to oxidative damage is party due to its high lipid content (Rice and Evans, 1993). Acetaldehyde toxicity impairs glutathione synthesis and causes lipid peroxidation, protein adduct formation, plasma membrane alterations and abnormal mitochondria oxidation of fatty acids (Amber, 2000). Significant decrease in the level of serum and hepatic lipid peroxidation as observed in groups pretreated with the extract of *Ziziphus mauritiana* is a clear indication that the extract contains antiperoxidative activity in the form of antioxidants thereby protecting the blood and liver against alcohol induced lipid peroxidation. The observed decrease in the level of serum vitamin C in alcohol control compared with normal rats was a clear indication that the antioxidant vitamin was used in combating the effect of oxidative stress due to lipid peroxidation. Increased levels of serum vitamin C in groups pretreated with 100 and 200 mg/kg body weight of ethanol extract of *Ziziphus mauritiana* leaf points to the rich antioxidant content of the plant extract which reduced the depletion of vitamin C in both serum and liver tissue. Dahiru *et al* (2005) had earlier reported anti-peroxidative activity of Ziziphus mauritiana extract in carbon tetrachloride-induced liver injury.

A further study is needed to isolate and characterize the active phytochemical principle responsible for the observed effect.

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